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ORIGINAL ARTICLE

Geographic variation in acoustic and visual cues and their potential to signal body condition in the Brazilian treefrog, *Boana albomarginata*

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Abstract
Anuran communication is largely based on acoustic signals, but different sensory modes are also widespread, including visual communication using body color traits as a way of signaling. The Brazilian treefrog, *Boana albomarginata*, has a complex behavioral repertoire presenting several call types and performing gestures as visual signals. This species has a greenish body color with orange patches on the flanks and thighs. These patches become visible when males are in a calling posture or performing visual signals such as leg kicking and limb lifting, suggesting that they might use the patches as visual cues. We sampled seven populations, using call recordings and photographs to access males call and color traits. We demonstrate that there is variation in color and call properties across populations. Additionally, we observe variation in the relationship between color traits and call properties in different populations, revealing that only two populations exhibit a significant correlation between color and call traits. Further, while call properties and color traits were not related with individual body size, they were associated with body condition. The results indicate a universal pattern across populations for call properties, wherein males in better condition consistently displayed lower-pitched calls, longer calls, and shorter intervals between calls. Regarding color traits, males in better condition in four out of the seven evaluated populations exhibited larger orange patch sizes, lower orange hue values, and higher hue contrasts. Although we observed some level of relation among color, call, and body traits, there is not a universal pattern across all populations.

Significance statement
Animal social interactions are mediated by signals transmitted through different sensory modes (i.e., acoustic, chemical, tactile, and visual), and more than one of these modalities can compose the behavioral repertoire of one species. Using photographs and acoustic recordings of *Boana albomarginata* males in natural environments, we documented geographic variation in both signals, call and color, and investigated their potential to convey individual body size and condition. Our findings reveal that both signals were correlated with individual body condition. In addition, color traits were associated with call properties in some populations.

Keywords Animal body color · Animal communication · Anurans · Bioacoustics · Visual cues · Visual communication

Introduction

Acoustic signaling is the predominant mode of communication used to mediate social interactions in anurans (Arch and Narins 2009; Chen and Wiens 2020), but they communicate using several other sensory modes [chemical (Poth et al. 2012), seismic/vibrational (Caldwell et al. 2010), visual (Augusto-Alves et al. 2018), and tactile signals (Augusto-Alves and Toledo 2022)]. Because light intensity contributes to the transmission of the visual signal, visual communication was linked mostly with diurnal species. However, anurans have well-adapted vision under low light intensity (Gomez et al. 2009, 2010; Kelber et al. 2017; Yovanovich et al. 2017; Robertson et al. 2022), which allows them to communicate by visual signals such as gestures, postures, or secondary visual cues [such as vocal sac

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color and movements during call activity (Starnberger et al. 2014; Jacobs et al. 2017)]. Frogs also have the capacity of color discrimination (Gomez et al. 2010; Kelber et al. 2017; Yovanovich et al. 2017; Robertson et al. 2022), permitting communication by body color traits (Vásquez and Pfennig 2007).

Regardless of the sensory modality (i.e., visual, auditory, etc.), communication often involves more than one behavioral component. In multicomponent communication/signals, only aspects of the same sensory modality are involved (Candolin 2003; Hebets and Papaj 2005; Partan and Marler 2005). For example, in a visual signal distinct body portions with distinct coloration traits may provide information for receivers (Ferrer et al. 2021), or in an acoustic signal, receivers may use temporal and spectral features to assess emitter traits (Vignal and Kelley 2007). There are differences on the propagation and perception of acoustic and visual signals, and sending the information by different sensory modes, i.e. multimodal communication, may be beneficial because it increases the probability of accurate information transfer. Acoustic signals can be transmitted over longer distances (Forrest 1994; Velásquez et al. 2018), and function in a variety of contexts, ranging from identification of conspecifics (Höbel and Gerhardt 2003; Köhler et al. 2017; Freitas and Toledo 2021), to assessment of the emitter's body physical state and position in short- and long-range interactions (Toledo et al. 2015; Köhler et al. 2017). Visual signals on the other hand are most effective at shorter distances, and function mainly for close-range assessment of the precise location and condition of the emitter (Giasson and Haddad 2007; Robertson and Greene 2017). Likewise, each communication mode has different challenges. Acoustic signals can be influenced by abiotic noise, such as wind, rain, flowing water (Lengagne and Slater 2002; Boeckle et al. 2009; Luther and Gentry 2013), anthropogenic noise (Parris et al. 2009), and intra- or interspecific biotic noise (e.g., frog choruses; Gerhardt and Klump 1988; Bee and Micheyl 2008). Similarly, detection of visual signals may be difficult in visually complex environments, such as windblown vegetation in the background or under insufficient illumination (Fleishman 1992; Ord et al. 2007; Ord and Stamps 2008).

Communication modes, either acoustic or visual signals, can vary between individuals and across populations. This geographic variability is often observed in anuran calls (Smith and Hunter 2005; Amezquita et al. 2009; Lee et al. 2016; Tessarolo et al. 2016), but also in the phenotype of body color and patterns (Amezquita et al. 2009; Robertson et al. 2009). Numerous hypotheses have been proposed to account for the geographic variation. The variation can arise from geographic history, sexual selection, genetic isolation, variations in environments, morphology, as well as countless others (Pröhl et al. 2007; Amezquita et al. 2009; Jang et al. 2011). Species with extensive distributions may encounter varying selective pressures. When coupled with geographic isolation, these pressures can lead to the manifestation of diverse patterns in their calls and colorations across populations. (Jang et al. 2011; Röhr et al. 2020).

Boana albomarginata is endemic to the Atlantic rainforest, widely distributed on the Brazilian costal region, and found on the mainland of the state of Paraíba (northernmost limit) to the state of Santa Catarina (southernmost limit) (Carnaval et al. 2009; Frost 2023), as well as some costal islands (Bittencourt-Silva and Silva 2013; Rebouças et al. 2020). The species has a greenish color pattern almost in the entire body, with exceptions of the flanks and thighs, which have orange patches (Fig. 1). They have a complex behavioral repertoire, comprising of different call types – 'advertisement call', 'distress call', and 'aggressive call' (Giasson and Haddad 2006; Toledo and Haddad 2009; Furtado and Nomura 2014) – and visual displays, including 'body raising' and 'limb lifting' (Hartmann et al. 2005; Giasson and Haddad 2006; Furtado and Nomura 2014) which expose the orange parts of the body that are generally hidden when the frogs are not active. These visual displays can be observed during close interactions between males or in the presence of nearby calling males, and they are linked with male-male agonistic interactions (Hartmann et al. 2005; Giasson and Haddad 2006).

Bright color patches on the thighs and/or flanks of cryptic frogs, which are only temporarily exposed, are frequently associated with defensive behaviors, acting as visual cues to alert predators (Williams et al. 2000; Toledo and Haddad 2009; Cannizzaro and Höbel 2023; Loeffler-Henry et al. 2023). Two observations suggest that *B. albomarginata*'s orange patches might also constitute a visual signal for conspecifics: first, there is sexual dimorphism, with males showing more pronounced coloration (Appendix I in

Fig. 1 Male of *Boana albomarginata*. Note the greenish color pattern almost in the entire body, with exceptions of the flanks and internal portion of the thighs. Photography by Luís Felipe Toledo

Supplementary Material). Second, visual displays that expose the orange patches are common during short-distance interactions with conspecifics (Hartmann et al. 2005; Giasson and Haddad 2006; Furtado and Nomura 2014).

Here, we conducted a multi-population study examining the call and color traits of the Brazilian treefrog *B. albomarginata* to elucidate the potential presence of geographic variation in these traits across populations. Furthermore, we predicted that the orange patches on the thigh are correlated with call properties and body measurements. In this way, both call and color might serve as honest signals of male quality in this treefrog.

Material and methods

Study site and data sampling

We sampled 170 *Boana albomarginata* males from seven sites in the Brazilian states of São Paulo (SP) and Rio de Janeiro (RJ), including mainland sites and islands (Fig. 2): Angra dos Reis (RJ, 22°56′12"S, 44°24′21"W; 29 males); Bertioga (SP, 23°48′55"S, 46°02′38"W; 30 males); Gipóia Island (RJ, 23°02′16"S, 44°21′19"W; 10 males); Itacuruçá Island (RJ, 22°56′20"S, 43°53′51"W; 13 males); Marambaia Island (RJ, 23°03′42"S, 43°59′01"W; 27 males); Picinguaba (SP, 23°21′35"S, 44°50′59"W; 30 males); and Seropédica (RJ, 22°45′05"S, 43°40′56"W; 31 males). We conducted one field expedition lasting 3–4 days for each locality between November 2018 and March 2019. Since our study involved focal animals in the field, it was not possible to record data blind.

We only sampled sexually mature males that were actively participating in a breeding chorus. Males were located through their call activity. First, we recorded the advisement call of each male for approximately three minutes (ZOOM H4n PRO and a Marantz PMD 661 MKII digital recorders with their built-in microphones). The recordings were made approximately 1 m away from the calling male, horizontally aligned with the individual, and under red lighting. Subsequently, we collected the individuals for measuring their snout-vent length (SVL; caliper 0.1 mm of precision), and weight (field scale 0.1 g of precision). These measurements were used to calculate the scaled mass index (SMI; Peig and Green 2009) as an indicator of body condition. Lastly, we photographed the frogs (Nikon D3200) dorsally, exposing the orange patches in the dorsal portion of the thighs. We took all photographs using a color standard grey 18% reflectance card in the background, for posterior light correction, and saved as a RAW file format. After that, the individual was toe-clipped for avoiding pseudoreplication and released at the same spot where it was captured. The toes clippings were preserved in 100% ethanol. Air temperature was measured during sampling with a HOBO data logger (Onset Computer Corporation).

Color analyses

Boana albomarginata individuals have orange patches in the femoral regions, contrasting with greenish body color (Fig. 3a). We characterized hue and relative size of the orange patches in relationship to the entire thigh. We imported all images into the ImageJ software (Schneider et al. 2012) with the plugin MICA Toolbox 2.2.2 (Troscianko and Stevens

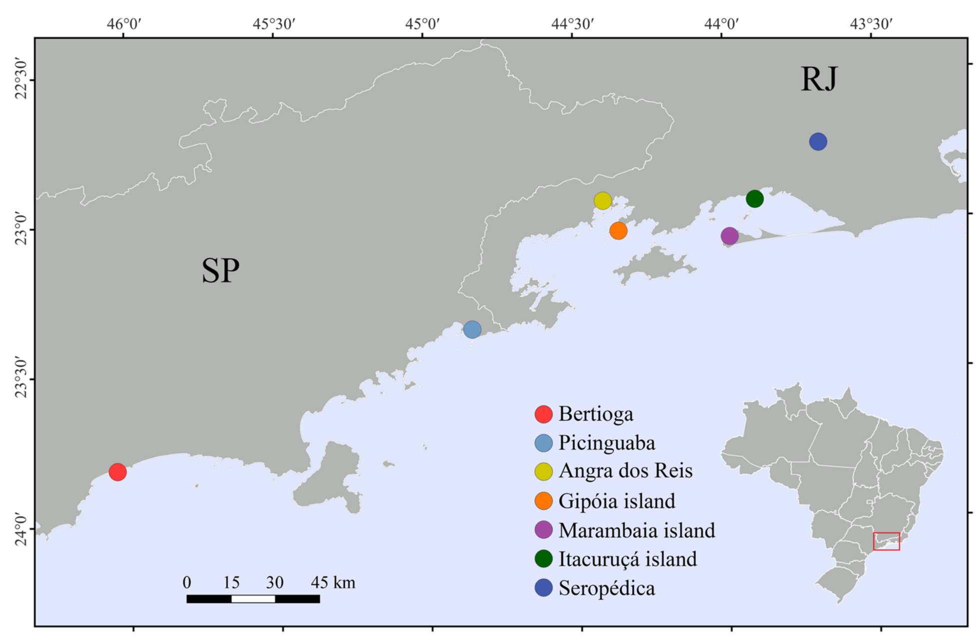


Fig. 2 Schematic map showing the distribution of the sampled populations in the present study. Fig. legend was organized geographically from the West to East

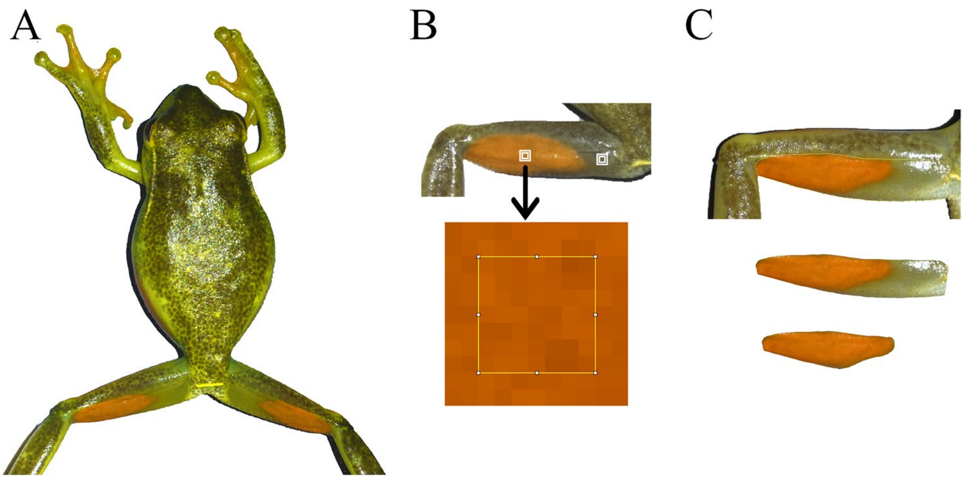


Fig. 3 **A** *Boana albomarginata* presents orange patches in the femoral regions that contrasts with greenish body color. **B** for hue analysis, we selected two regions of interests (ROIs—white squares: one in the middle of the orange patch; and another out of the patch, in the leg green portion). Each selected ROI had 7^2 pixels, of which, we extracted red–green–blue reflectance information. **C** demonstration of how we calculated the relative size between thigh and orange patch. We selected the thigh portion that contains the orange patch, after, we selected the orange patch (relative size was calculated by dividing the total thigh area by the patch area and multiplied by 100)

2015). For each photograph, we generated a Multispectral Image calibrated (.mspec) based on the standard grey card reference (Troscianko and Stevens 2015). Then, we selected two 7^2 pixels as our regions of interests (ROIs), being one in the middle of the orange patch, and another out of the patch, in the leg green portion (Fig. 3b). From the ROIs we accessed reflectance in the red–green–blue (RGB) color system, on the scale of 0–255 for each channel, and RGB were used to obtain hue values. We calculated the hue contrast (ΔH) of the orange patch (H_o) related to the thigh green portion (H_g) as: $\Delta H = (H_o - H_g)/(H_o + H_g)$ [based on a 'brightness contrast' calculation (Fleishman and Persons 2001)]. We also used ImageJ to determine the relative size of the orange patch. First, carefully, we selected all the thigh contour and measured the number of pixels of the selection, subsequently, we did the same procedure with the patch (Fig. 3c). To calculate the relative size of the orange patch, we divided the patch area by the total thigh area and multiplied by 100.

Audio analyses

Prior to audio analyses, we standardized all recordings at a sampling rate of 44.1 kHz, and resolution of 16 bits, and normalized (peak -1 dB), using Adobe Audition 2020. We analyzed these calls in Raven 1.6.1 PRO (Center for Conservation Bioacoustics 2019), with the following configuration: 60% contrast, 60% brightness, 512 DFT, window type Hann. Individuals emit advertisement calls as simple units (single calls) or in call series of two or more calls rhythmically emitted (Giasson and Haddad 2006; Fig. S1). In order to standardize we only included single calls in this study [call-centered approach as indicated (Köhler et al. 2017)]. We analyzed on average (± SD) 8.26 ± 2.4 (2–10) calls per individual (Table S1). During the acoustic analyses, we noticed that the call harmonic which contains the dominant frequency (frequency with the highest sound energy) changes according with the analyzed call; this happens among different individuals, but also among different calls from same male. Thus, for spectral analysis we selected separately the two harmonics, instead of one selection for the entire call (Fig. S1 shows details of the selection limits). We characterized the calls by the following properties: call duration; pulses per call; intercall interval; call period (the call and the silence period subsequently); first harmonic dominant frequency (peak frequency function in Raven); first harmonic minimum frequency (frequency 5% function in Raven); first harmonic maximum frequency (frequency 95% function in Raven); second harmonic dominant frequency; second harmonic minimum frequency; second harmonic maximum frequency; dB difference (second harmonic dB FS – first harmonic dB FS; delta power dB FS function in Raven). Several temporal properties were temperature-dependent, and therefore, values for these traits were temperature corrected to 25.4 °C (mean temperature of our sample; Table S2 for temperature information for each population) using the results of a linear regression of the call variable on temperature before further statistical analyses. Using the same method, we corrected the spectral properties of the call based on the body size of the individuals.

Statistical analyses

Because traits constituting a signal (color or call, respectively) are frequently correlated, we conducted a Principal Component Analysis (PCA) to generate smaller numbers of uncorrelated variables. For color, we conducted a PCA that included four color traits (orange ROI hue, green ROI hue, contrast hue, and patch relative size). For advertisement calls, we conducted another PCA that included 11 call traits (call duration, pulses per call, intercall interval, call period, first harmonic dominant frequency, first harmonic minimum frequency, first harmonic maximum frequency, second harmonic dominant frequency, second harmonic minimum frequency, second harmonic maximum frequency, and dB difference). We used the resulting PC scores in subsequent analyses. We ran all analyses using JMP PRO v. 11.1.0 (SAS Institute Inc., 2015). We built the Figs in R version 3.6.3 (R Core Team 2019).

Color and call geographic variation To test for geographic variations in color and call traits between *Boana albomarginata* populations, we conducted nested analyses of variance (ANOVA) using the products from both PCAs (color and call, respectively), nested by population. Then, we ran post hoc Tukey tests.

Relationship between patch color and call traits We used standard least squares models to assess the relationship between the orange color patch and call traits; we focused on the orange leg patch because we did not expect the green based color of the frogs to act as a potential visual signal. We fitted the models with the data of the principal component summarizing variation in the orange leg patch ($PC1_C$) as response. As predictor we included the principal component summarizing variation in calls [either spectral ($PC1_A$) or temporal ($PC2_A$)], population, and call*population (either $PC1_A$*population or $PC2_A$*population).

Relationship of color patch and call traits with male body measures We used standard least squares models to assess the relationship of putative signal traits (orange color patch and call traits, respectively) with body length (SVL) and body condition (SMI). We fitted different models using orange patch traits ($PC1_C$ from color PCA), spectral ($PC1_A$ from advertisement call PCA), and temporal ($PC2_A$ from call PCA) properties as response variables, and SVL, SMI, population, SVL*population, SMI*population as predictors, in all models. We included both SVL and SMI in the same model, since they are uncorrelated descriptors ($|r| < 0.7$) of male morphology and energy reserves.

Results

The PCA of color traits returned two principal components with eigenvalues greater than 1 ($PC1_C = 2.17$, $PC2_C = 1.21$). Together they account for 84.5% of the variation. $PC1_C$ loaded primarily with relative patch size, orange hue and contrast hue. $PC2_C$ loaded primarily with green hue (Table 1).

Table 1 Factor loading on the first two principal components, which together are responsible for 84.5% of variation in *Boana albomarginata* color traits

factor	$PC1_C$	$PC2_C$
patch relative size	-0.4674	-0.2831
hue orange	0.6286	0.1946
hue green	-0.0638	0.8826
hue contrast	0.6183	-0.3208

Table 2 Factor loading on the first two principal components, which together are responsible for 70.7% of variation in *Boana albomarginata* advertisement call properties

factor	$PC1_A$	$PC2_A$
call duration	0.10942	-0.401
pulses per call	0.14546	-0.3589
call period	-0.0888	0.50259
intercall interval	-0.0885	0.5043
dB difference	0.17796	-0.3068
first harmonic minimum frequency	0.36941	0.19826
first harmonic maximum frequency	0.39272	0.1298
first harmonic dominant frequency	0.38439	0.18639
second harmonic minimum frequency	0.39715	-0.0598
second harmonic maximum frequency	0.39383	0.10036
second harmonic dominant frequency	0.41011	0.06912

The PCA of advertisement call traits returned two principal components with eigenvalues greater than 1 ($PC1_A = 5.37$, $PC2_A = 2.43$). Together they account for 70.7% of the variation. $PC1_A$ loaded primarily with spectral properties (first harmonic dominant frequency, first harmonic minimum frequency, first harmonic maximum frequency, second harmonic dominant frequency, second harmonic minimum frequency, and second harmonic maximum frequency). $PC2_A$ loaded primarily with temporal and power properties (call duration; pulses per call; intercall interval; call period; dB difference) (Table 2).

Geographic variation in color and advertisement call properties

In total, we evaluated 170 *Boana albomarginata* males from seven sites (see Table S3 for mean values for color, call and body size/condition traits for each population). We document significant geographic variation in all color and call traits: Orange patch size and color ($PC1_C$: $F_{(6,146)} = 8.11$,

$P < 0.001$; Fig. 4a); green body portion (PC2$_C$: $F_{(6,146)} = 6.93$, $P < 0.001$; Fig. 4b); spectral advertisement call properties (PC1$_A$: $F_{(6,161)} = 18.02$, $P < 0.001$; Fig. 4c), and temporal advertisement call properties (PC2$_A$: $F_{(6,161)} = 3.33$, $P = 0.004$; Fig. 4d).

Relationship between orange patch color and call traits

At the species level, we detected an association between the orange patch traits and temporal call properties (Table 3; bottom) but not spectral call properties (Table 3; top). There was also substantial geographic variation in advertisement call properties, as well as in the relationship between the orange color patch and advertisement call properties, indicted by significant call (PC1$_A$ or PC2$_A$)*population interaction terms (Table 3).

The slopes of the regressions between spectral call properties and orange color patch varied between—0.40 and + 0.76 for different populations (Fig. 5a, Table S4). A population-based post-hoc analysis showed that the relationship was statistically significant for two populations: Picinguaba ($r^2 = 0.31$, $P = 0.002$) and Seropédica ($r^2 = 0.22$, $P = 0.007$); both showed positive slopes indicating that males with lower-frequency calls had more pronounced

Table 3 Results of the standard least squares models showing the relation of orange patch traits (loaded in the PC1$_C$ of the PCA with color traits—patch relative size, orange hue, and contrast hue) with acoustic properties. PC1$_A$ of the PCA with call traits was loaded by spectral properties (first harmonic dominant frequency, first harmonic minimum frequency, first harmonic maximum frequency, second harmonic dominant frequency, second harmonic minimum frequency, and second harmonic maximum frequency) and PC2$_A$ with temporal properties (call duration; pulses per call; intercall interval; call period, and dB difference). Bold font indicates significant *P*-values

Orange patch color traits (PC1$_C$)				
factor	**DF**	**Sum of squares**	**F**	***P***
PC1$_A$ (Spectral)	1,6	1.779	1.275	0.261
population	1,6	21.811	2.605	0.02
PC1$_A$*population	1,6	33.052	3.948	0.001
Orange patch color traits (PC1$_C$)				
factor	**DF**	**Sum of squares**	**F**	***P***
PC2$_A$ (Temporal)	1,6	8.131	5.284	0.023
population	1,6	82.317	8.916	<0.001
PC2$_A$*population	1,6	24.744	2.68	0.017

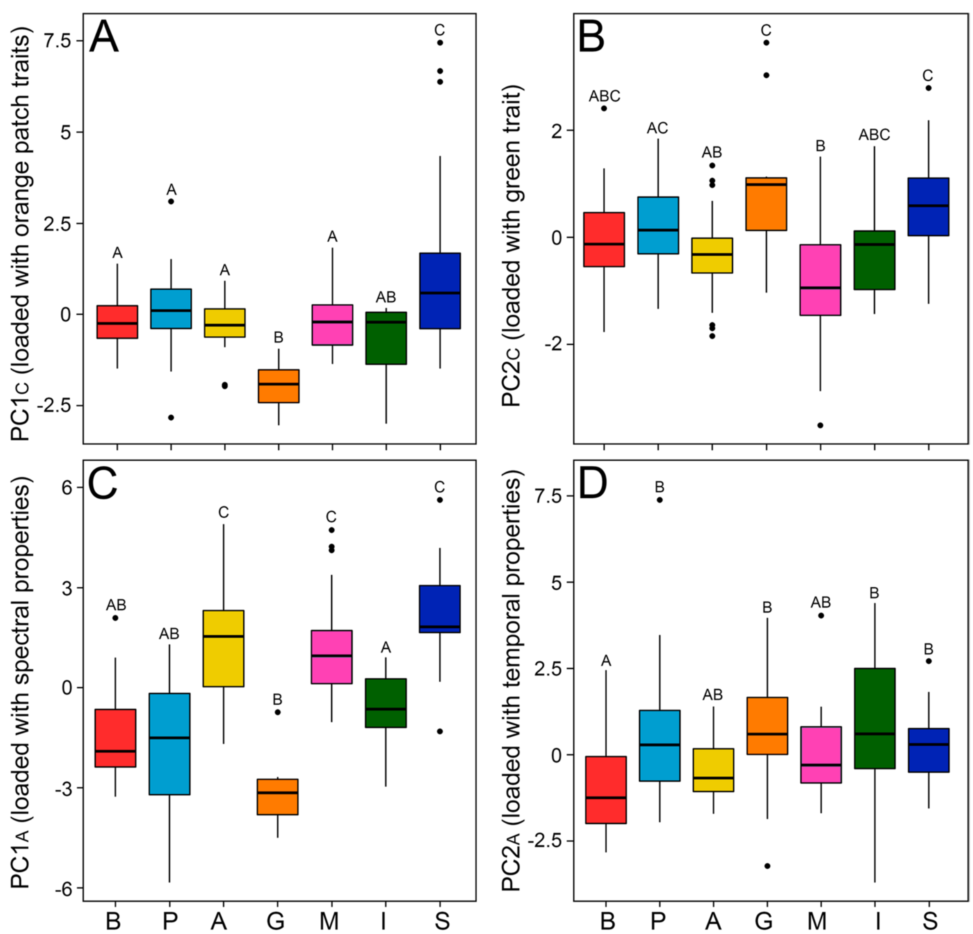


Fig. 4 Geographic variation in color and call traits. **A** orange patch traits (PC1$_C$ of the PCA set with color traits). **B** green body portion trait (PC2$_C$ of the PCA set with color traits). **C** spectral properties (PC1$_A$ of the PCA set with call proprieties). **D** temporal properties (PC2$_A$ of the PCA set with call proprieties). Boxplots indicate median, upper, and lower quartiles, upper and lower whiskers enclose the data range, excluding outliers. Boxplot was ordered geographically from the westmost to eastmost population. Abbreviation of populations: B = Bertioga; P = Picinguaba; A = Angra dos Reis; G = Gipóia Island; M = Marambaia Island; I = Itacuruçá Island; S = Seropédica

orange patches (larger relative size, higher contrast hue value, and lower orange hue value). On the other hand, no relationship between orange patches and spectral properties was observed for the other five populations (All $P > 0.05$).

The slopes of the regressions between temporal call properties and orange color patch varied between—0.16 and + 0.96, with most populations showing relatively flat slopes (Fig. 5b, Table S4). This model returned a significant call term (Table 3), suggesting a general pattern of association between the temporal call properties of the advertisement call and the orange patch. However, visual inspection of the associated graph (Fig. 5b) suggests that this result is likely due to one population with very steep slope (other populations showed relatively flat slopes). In fact, the population-based post-hoc analysis showed that the regression was statistically significant only for one population: Seropédica ($r^2 = 0.17$, $P = 0.02$). Here, males with longer duration/more pulses per call, and shorter intervals between calls had more pronounced orange patches (larger relative size, higher contrast hue value, and lower orange hue value). For the other six populations, we did not find relationship between orange patches and temporal properties (All $P > 0.05$).

Relationship of color patch and call traits with male body measures

Both spectral and temporal call properties were associated with body condition (SMI; Table 4): males with better body condition had lower frequency calls (Fig. 6a), longer duration/more pulses per call, and shorter intervals between calls (Fig. 6b). There was geographic variation in call properties and a significant effect of population (Table 4). By contrast, body length (SVL) was not associated with advertisement call traits (Table 4). Orange patch size and color was also associated with body condition: males in better condition had more pronounced orange patches (larger relative size, higher contrast hue, and lower orange hue) (Table 4; Fig. 6c). Again, there was geographic variation in how closely the orange patch was associated with body condition (Table S6). The slopes of the regressions between orange color patch and body condition varied between – 2.64 and + 0.52. Most populations showed negative slopes (Fig. 6c; Table S7), and the regression was statistically significant for 4 populations: Itacuruçá island ($r^2 = 0.64$, $P = 0.005$); Marambaia island ($r^2 = 0.17$, $P = 0.034$); Picinguaba ($r^2 = 0.31$, $P = 0.002$); Seropédica ($r^2 = 0.39$, $P < 0.001$); other three populations $P > 0.05$. By contrast, body length (SVL) was not associated with orange patch traits (Table 4).

Discussion

We conducted a multi-population study examining call and color traits of the Brazilian treefrog *Boana albomarginata*. Consistent with the pattern observed in the geographic variation of their advertisement call frequencies (Rebouças et al. 2020), we also document substantial geographic variation in advertisement call temporal properties and body

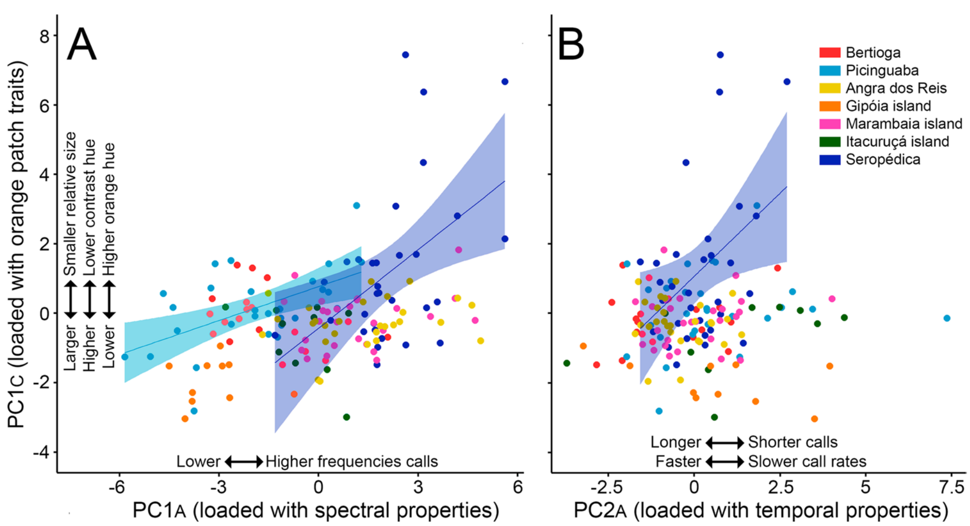


Fig. 5 Relationship between color and call traits. **A** In two populations spectral call traits and orange color traits were related; males with more pronounced color patches (lower $PC1_C$ scores) had lower frequency calls (lower $PC1_A$ scores). **B** In one population temporal call traits and orange color traits were related; males with more pronounced color patches (lower $PC1_C$ scores) had longer calls/more pulses per call, and shorter intervals between calls (lower $PC2_A$ scores). In Seropédica we observed some individuals with exceptionally high color PC sores. To examine whether they biased our results, we ran the analyses with (Table 3) and without (Table S5) them. Results were very similar, and accordingly, we present data from the entire dataset. Fig. legend was organized geographically from the westmost to eastmost population

Table 4 Results of the standard least squares models showing the relation of signals with males' traits. Abbreviations: SVL – Snout-Vent Length; SMI – Scaled Mass Index. Bold font indicates significant *P*-values

Call spectral properties ($PC1_A$)				
factor	DF	Sum of squares	F	*P*
SVL	1,6	1.584	1.114	0.293
SMI	1,6	32.913	23.156	< 0.001
population	1,6	103.133	12.094	< 0.001
SVL*population	1,6	12.902	1.513	0.178
SMI*population	1,6	12.716	1.491	0.185
Call temporal properties ($PC2_A$)				
factor	DF	Sum of squares	F	*P*
SVL	1,6	3.584	1.779	0.184
SMI	1,6	19.233	9.548	0.002
population	1,6	63.954	5.291	< 0.001
SVL*population	1,6	24.813	2.053	0.062
SMI*population	1,6	7.324	0.606	0.725
Orange patch color traits ($PC1_C$)				
factor	DF	Sum of squares	F	*P*
SVL	1,6	0.548	0.483	0.488
SMI	1,6	10.694	9.422	0.003
population	1,6	13.75	2.019	0.067
SVL*population	1,6	5.963	0.876	0.515
SMI*population	1,6	25.024	3.675	0.002

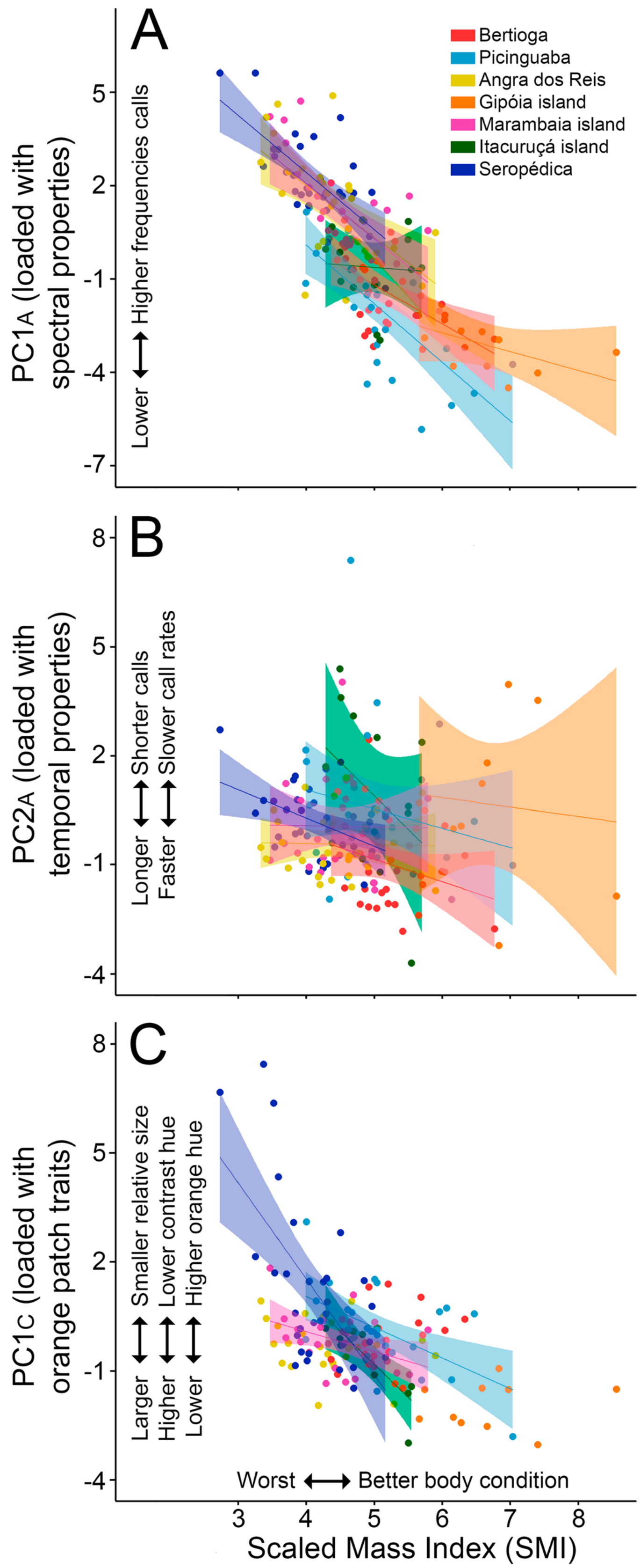
color traits across populations (Fig. 4). Geographic variation in call and color traits is widely reported for anurans (Summers et al. 2003; Heyer and Barrio-Amorós 2009; Zornosa-Torres and Toledo 2019; Augusto-Alves et al. 2020; de Souza et al. 2021). For instance, color pattern polymorphisms were observed in two Central American species, *Dendropsophus ebraccatus* and *Agalychnis callidryas*, demonstrating significant geographic variation across populations throughout the regions of Costa Rica and Panama (Robertson et al. 2009). In another example, *Dendropsophus cruzi* exhibits call variation in spectral and temporal properties across populations, with dominant frequency, call duration, and rate showing a negative correlation with latitude (Tessarolo et al. 2016). Several reasons have been suggested for the inter-population variation, including influence of climate, morphology, geographic and genetic isolation, population density, vegetation and community structure (Pröhl et al. 2007; Rudh et al. 2007; Robertson et al. 2009; Forti et al. 2016; Köhler et al. 2017; Serrano et al. 2020; Fernandes et al. 2021; da Rosa et al. 2023).

The pigment categories of carotenoids and pteridines are responsible for producing yellow-orange-red coloration in vertebrates (Mills and Patterson 2009; Weiss et al. 2012; Umbers et al. 2016; Merkling et al. 2018), including frogs (Suga and Munesada 1988; Umbers et al. 2016; Brenes-Soto et al. 2017). Unlike pteridines, that are synthesized during purine production (Ziegler 2003; Le Guyader et al. 2005), carotenoids have to be acquired from the diet (Feltl et al. 2005; Umbers et al. 2016). Carotenoid-supplementation studies with captive frogs support this link between diet and color (Brenes-Soto and Dierenfeld 2014; Umbers et al. 2016). For instance, *Pseudophryne corroboree* individuals fed with carotenoid-poor diet still developed the species yellow-black patched pattern, but individuals fed with a diet supplemented with carotenoids presented patches more orange (yellow in the poor dietary), and significant difference in chroma (higher saturation) (Umbers et al. 2016). In this manner, both the observed variation in orange patch among populations (Fig. 4) and the differences in how these patches are associated with body condition (Fig. 6, Table 4) may be outcomes related to dietary quality and availability in the different studied sites.

Acoustic allometry describes how information about body size is conveyed by acoustic signals (Ryan 1988; Fletcher 2004), and the pattern where larger body size is associated with lower-frequency calls is widely present across anuran taxa (Gingras et al. 2013; Tonini et al. 2020; Augusto-Alves et al. 2021). Yet, exceptions from this rule have been documented (Tonini et al. 2020), including some populations of *B. albomarginata* (Rebouças et al. 2020), and a study involving a range of acoustically communicating taxa concluded that most acoustic signals do not appear to have been selected to function as indicators of body size (Rodríguez et al. 2015). Our study corroborates the previously described frequency escape of the spectral acoustic allometry in *B. albomarginata* and extends it to include temporal properties and orange patch traits which were also not predictors of male size in all populations.

On the other hand, we did find that calls (both spectral and temporal properties) and sometimes also color traits were associated with SMI. Therefore, both color and calls potentially serve as indicators of male body condition for conspecifics. Aggressive interactions between males of *B. albomarginata* involve aggressive calls combined with visual signals (leg kicking, limb lifting and body raising: Giasson and Haddad 2006), some of which increase visibility of their orange color patches. We do not have information about the use of visual signaling/exposure of orange patches during courtship, but the elevated stance of calling males exposes those patches and allows for inspection by mate-searching females. Evaluating the traits of orange patches between unmated and mated males could provide new evidence of the use of color traits in the mate choice process, if the traits between groups differ. However, to examine the relative importance of call and color signals during male competition, courtship, and mate choice in *B. albomarginata*, behavioral experiments will be necessary. Furthermore, based on these behavioral experiments, future studies could address how these different communication modes are related in a multimodal context or if they are completely independent within the behavioral repertoire.

Fig. 6 Relationship between color and call traits with body condition (SMI: Scaled Mass Index). **A** Males with better body condition (higher SMI) had lower frequency calls (lower $PC1_A$ scores). **B** Males with better body condition (higher SMI) had longer and faster call rate (lower $PC2_A$ scores). **C** In 4 populations, males in better condition (higher SMI) had more pronounced orange patches (lower $PC1_C$ scores). Trend line and 95% confidence intervals indicate populations with significant relationships. Fig. legend was organized geographically from the westmost to eastmost population



Conclusion

Based on a multi-populational survey, we demonstrated that the Brazilian treefrog *Boana albomarginata* exhibits geographic variation in both color and call traits. Additionally, we found that information about body condition may potentially be transmitted through different components within the same communication modality, the spectral and temporal properties of the calls. Furthermore, we observed an association between body color traits and body condition in some populations, indicating that orange patches may also convey information about males' body condition. Behavioral experiments will be necessary to test whether males and/or females attend to variations in orange patch color to mediate different social contexts.

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Author contribution GA-A and LFT conceptualized the study and experimental design. GA-A collected the data, and analyzed the photographs and recordings. GA-A and GH conducted the statistical analyses. GA-A prepared the manuscript drafts, and GH and LFT critically reviewed and edited subsequent versions. All authors have read and approved the final manuscript and submission for publication.

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Data availability Toes clippings were deposited in the TLFT tissue collection at Universidade Estadual de Campinas (Unicamp; TLFT 4501–609; 4611–8; 4620–4; 4626–73). Audio files were deposited at Fonoteca Neotropical Jacques Vielliard (FNJV), Museu de Diversidade Biológica (MDBio), Unicamp (FNJV 43831–939; 43,941–53; 43,955–83; 43,985–4003), following previous recommendations (Dena et al. 2020). The datasets generated and analyzed during the current study are available as supplementary material.

Declarations

Ethical approval The use of animals in this study adheres to the set forth by the Animal Behavior Society/Association for the Study of Animal Behaviour. This study was approved by the Instituto Chico Mendes de Conservação da Biodiversidade (SISBio #63697–2), Comissão Técnico-Científica, Instituto Florestal (COTEC #483/2018), ethics committee of Unicamp (Comissão de Ética no Uso de Animais, CEUA #4983–1/2018), and was registered at the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SISGen #AE9A0E0).

Conflict of interest The authors declare no competing interests.

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